by monovalent cations sodium (Na^+) and potassium (K^+). The structure of G-quadruplexes can be highly polymorphic. H-Tel, an ODN with four consecutive repeats of the human telomeric sequence, [d(TTAGGGTTAGGGTTAGGGTTAGGG)], can assume different monomolecular G-quadruplex topologies depending on the type of cation present in solution. The loop sequences between the guanine repeats can also affect the conformation of the G-quadruplex formed. The biological relevance of these investigations arises from the implication of G-rich sequences in diseases so that G-quadruplexes may be drug targets in cancer and other diseases. Our lab has shown that at high concentrations, folded H-Tel self-associates to form multi-molecular species. Using H-Tel and H-Tel derivatives, we are studying the characteristics of G-quadruplexes they form and the conditions under which the folded species self-associate. The structure and energetic properties are studied as functions of the type of cation, the concentration of the cation, and the sequence of the bases in the loops between the tetrad-forming guanines. Our studies use circular dichroism spectroscopy (CD), UV spectroscopy, differential scanning calorimetry (DSC), and gel electrophoresis.

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Microheterogeneity of Telomeric DNA Guanine Residues: pH Dependent Spectroscopic Studies of Fluorescently Labeled Model Trinucleotides

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Guanine-rich telomeric DNA $(TTAGGG)_4$ (hT_4) folds through Hoogsteen base pairing to form G-quadruplex structures, which inhibit the activity of telomerase, a key enzyme in tumorigenesis. Using a fluorescent guanine analog, 6-methylisoxanthopterin (6MI), we previously observed varying fluorescence intensity and emission wavelength shifts when incorporated into selective quadruplex sequence guanine positions, either in the folded or unfolded conformations. Our data suggest that quadruplexed guanine residues may play differing roles in maintaining global conformational stability through varying base-base, hydrogen bonding and/or solvent interactions. Previously long-wavelength emission spectral shifts of 6MI (*Hawkins et al., Analytical Biochemistry (1997) 244, 86*) were proposed to arise from possible deprotonation of 6MI at position N3, a key residue involved in Hoogsteen base-pairing. Here the effect of pH on the fluorescence properties of 6-MI (F) has been studied, when incorporated into model trinucleotides of hT_4 with varying flanking bases: -AFG-; -GFG-; -GFT-; and -GGF-. All 6MI trinucleotides showed intensity quenching over 6MI, with a greater effect for purine neighbors (GFG>GGF>GFT>AFG) as noted previously (*Poulin et al., Biochemistry (2009) 48, 8861*). Increasing pH resulted in fluorescence intensity quenching for the parent 6-MI and a red wavelength fluorescence shift (excited-state pK_a ~8.3). Interestingly fluorescence lifetime values appeared invariant over the corresponding pH range (τ_{av} ~ 6.2ns at pH 5 and ~6.8ns at pH 11). Absorption spectral pH titrations revealed a ground-state pK_a for 6MI of ~9.8. Model 6MI labeled trinucleotides also revealed long-wavelength emission shifts, but associated with a dequenching of fluorescence signals. Increased excited-state pK_a values corresponded closer to ground state values for the fluorescent trinucleotides. Effects of microheterogeneity on possible deactivating pathways for 6MI labeled trinucleotides, including possible electron transfer, deprotonation, and quenching mechanisms will be discussed. ([1]Supported by NIH 5SC3 GM095437).

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Polyelectrolyte Effects in G-Quadruplexes

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The role of counterion condensation as a dominant force governing the stability of DNA duplexes and triplexes is well established. In contrast, the effect of counterion condensation on the stability of G-quadrupex conformations is poorly understood. Unlike other ordered nucleic acid structures, G-quadruplexes exhibit a specific binding of counterions (typically, Na^+ or K^+) which are buried inside the central cavity and coordinated to the O6 carbonyls of the guanines forming the G-quartets. While it has been known that the G-quadruplex-to-coil transition temperature, T_M, increases with an increase in the concentration of the stabilizing ion, the contributions of the specific (coordination in the central cavity) and nonspecific (condensation) ion binding have not been resolved. In this work, we separate the two contributions by studying the change in T_M of preformed G-quadruplexes following the addition of non-stabilizing ions Li^+, Cs^+, and TMA^+. In our studies, we used two G-quadruplexes formed by the human telomeric sequences which are distinct with respect to the folding topology and the identity of the stabilizing ions. Our data suggest that the predominant ionic contribution to G-quadruplex stability comes from the specifically bound Na^+ or K^+ ions and not from counterion condensation around the DNA. We offer molecular rationalizations of the observed insensitivity of G-quadruplex stability to counterion condensation and emphasize the need to expand such studies to assess the generality of our findings.

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Structural Dynamics and Polymorphism of Telomeric G-Quadruplex DNA Structures

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Telomeres protect the ends of chromosomes and are important for genomic stability. Telomeric DNA is double stranded with a single strand overhang and contains the same sequence repeated over and over. This G rich DNA can fold into G-quadruplex structures, which can contribute to the regulation and maintenance of telomere length. Telomeric G-quadruplex structures, containing the human telomeric repeat TTAGGG, fold under many different conformations depending on their environment. They show large conformational diversity in solutions containing KCl and the different conformations interconnect dynamically one with another with time constants that depend on salt concentration. We investigate here different telomeric G-quadruplex structures using single molecule FRET microscopy to obtain a direct insight into their structural polymorphism and dynamics. Experimental conditions are carefully chosen and adjusted to yield G-quadruplex foldings with both large and low conformational heterogeneity. This study allows a clear identification of FRET efficiency signatures for different G-quadruplex conformations.

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Kinetics of Two Slow Conformational Transitions of the Quadruplex Structure of the Thrombin Binding Aptamer and their Potassium Dependence

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The thrombin binding aptamer, TBA, forms an anti-parallel chair type quadruplex structure that has been found to have at least two slow conformational transitions. One of these involves the loop residues T4 and T13 that hydrogen bond to each other and stack with the G2-G5-G11-G14 quartet. This conformational transition appears to be between the stacked and unstacked states of T4 and T13. There are both enthalpic and entropic contributions to this transition barrier. The rate of this transition decreases with increasing potassium concentration in agreement with the model that has potassium stabilizing the stacking of T4 and T13. The other slow transition involves at least the G1 and G14 residues that are in quartets. The barrier to this transition is almost entirely entropic and the rate is not particularly sensitive to the concentration of potassium in the range of 10 to 40 mM. These two transitions have rates in the hundreds of millisecond range and can be monitored by standard NMR methods. These transitions offer insights into the properties of TBA as well as providing a model system for investigation of quadruplex structural transitions under a range of experimental conditions as well as validating models of the transitions.

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Z-DNA-Forming TG Repeats are Dynamic Mechanical Switches Sensitive to Tension and Torsion

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Left-handed Z-DNA has been implicated in various biological processes. Physical basis of functions of Z-DNA however remains elusive. Despite the prevalence of alternating thymine-guanine (TG) repeat sequences in eukaryotes, the Z-DNA formed by these sequences has remained incompletely understood. Here we investigated mechanics and kinetics of the B-Z transition occurring in short TG repeat sequences under mechanical controls. We discovered that the sequences switch dynamically between B- and Z-DNA states and the B-Z transitions in those sequences are sensitive to applied tension and torsion. By monitoring the B-Z transition in real time, the transition rates were directly measured and were of the order of 1 Hz.